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Thiol /Disulphide homeostasis in children with acute pyelonephritis and cystitis

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Abstract
Aim: Urinary tract infection is frequently seen in children, and in recurrent cases, invasive methods such as scintigraphy may be required for prognosis. We aimed to investigate whether the thiol/disulfide homeostasis has any role or effect on acute pyelonephritis and cystitis in pediatric patients.
Material and Methods: This case-control study was collected between June 2016 and June 2019. A total of 80 children (aged between 6 months and 17 years) participated in the study. Patient (80 children) demographics, serum and urine analyzes were collected.
Results: A significant difference was found when ages, heights, weights, body temperature, diastolic blood pressure, hemoglobin, leukocyte, C reactive protein (CRP), urine analysis and albumin values were compared. No significant difference was observed in systolic blood pressure, platelets and in thiol, disulfide and ischemia modified albumin (IMA) values and thiol/disulfide related ratios when all groups were compared. We also could not found any correlations results between thiol/disulfide and other parameters.
Discussion: Although there are meaningful results in many studies about thiol/disulfide homeostasis, we could not achieve the same results for urinary tract infection in our study. The pathophysiology of acute pyelonephritis and cystitis might not be related to the thiol/disulfide mechanism or does not cause enough change in the values of these parameters in the blood.

Keywords
Thiol/Disulfide; Pyelonephritis; Cystitis

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Introduction

Urinary tract infection is one of the most common bacterial infections in children. When the infection is in the upper urinary tract, it is defined as pyelonephritis; in the lower urinary system, it is called cystitis if appropriate and adequate treatment is not performed [1-3]. Although acute pyelonephritis is common in children nowadays, Technetium-99m-dimercaptosuccinic acid (DMSA), which is an invasive examination for the child, is used to determine the prognosis and renal scar, diagnosis can lead to serious consequences, such as chronic pyelonephritis, hypertension and renal failure [4].

Oxidative stress plays an important role in the etiopathogenesis of this disease, and there are experimental studies in the literature about the protective properties of some antioxidants [5]. Oxidative stress is caused by the deterioration of the balance between antioxidant molecules and reactive oxygen species (ROS) [6]. ROS above the physiological level causes oxidation of many molecules, such as radical-based cysteine residues. As a result of this reaction, the sulfur atom present in the cysteine side chain is oxidized to disulfide [7]. In this case, the dynamic thiol/disulfide balance shifts to the disulfide form.

Thiol/disulfide homeostasis plays a very important role in maintaining physiological processes such as antioxidant defense, apoptosis and protection of protein structures necessary for the organism [8]. In degenerative diseases such as diabetes, cardiovascular diseases, and rheumatic diseases, this balance is thought to shift in favor of disulfide [9]. It is not known how the balance of thiol/disulfide changes in acute pyelonephritis with high morbidity.

A simple, reliable and precise measurement method was developed by Erel et al. in 2014 to measure thiol/disulfide homeostasis, native thiol and total thiol levels for the first time [9]. In this study, we aimed to investigate the oxidative thiol/disulfide homeostasis in pediatric patients with pyelonephritis and cystitis attacks compared to healthy controls. We foresee that thiol/disulfide homeostasis may be a predictor of oxidative stress in the near future and may indicate the severity of the disease and the degree of recovery after treatment. We anticipate that our study may contribute to fill the gap in the literature on this subject.

Material and Methods

Study population

This case-control study was collected between June 2016 and June 2019. A total of 80 children (aged between 6 months and 17 years) participated in the study. The control group consisted of 40 healthy children without any disease who were to undergo circumcision, inguinal hernia or frenilum linguale operations in the Department of Pediatric Surgery, Trabzon, Turkey. Twenty pediatric patients with pyelonephritis and 20 pediatric patients with cystitis in the Department of Pediatric Nephrology, Trabzon, Turkey were included as the patient groups. The study was approved by the Local Ethics Committee and informed consent was obtained from the parents/caregivers of the patients.

Procedures

Patient demographics, sex, age, height, weight, body temperature and systolic/diastolic blood pressures were collected.

Laboratory method

Urine samples for urinalysis and urine culture were obtained using catheterization from non- continent patients, and clean middle-flow urine was obtained from continent children. Urine samples were taken for analysis (leukocyte, nitrite and protein) with a Beckman Coulter autoanalyzer. In the urinary culture, ≥10,000 colonies of a single pathogen were considered significant from non-continent patients; ≥100,000 colonies of a single pathogen were considered significant from continent patients.

Venous blood samples were taken to measure hemoglobin, leukocyte, platelets and determined with a Sysmex autoanalyzer. C reactive protein (CRP) was determined with a Beckman Coulter autoanalyzer. Additional 2-3 cc blood samples were taken to measure total thiol, native thiol, disulphide levels, disulphide/native thiol, disulphide/total thiol, native thiol/total thiol ratios, ischemia modified albumin (IMA) and albumin at Ankara Ataturk Education and Research Hospital using the method described by Erel et al [9]. Sera were separated after centrifugation at 1600 g for 10 minutes, and samples were transferred to Eppendorf tubes and stored at -80ºC.

Thiol- disulphides homeostasis parameters measurement:

Thiol/Disulphide Homeostasis tests were measured by an automated spectrophotometric method described by Erel & Neselioglu [9]. For short, the disulphide bonds were first reduced to form free functional thiol groups with sodium borohydride. Unused reductant sodium borohydride was consumed and removed with formaldehyde to prevent reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic) acid), and all of the thiol groups, including reduced and native thiol groups, were determined after the reaction with DTNB. Half of the difference between total thiols and native thiols provides a dynamic disulphide amount. After the determination of native and total thiols, and disulphide amounts, the disulphide/total thiol (SS/SH+SS), disulfide/native thiol ratio (SS/SH) and native thiol/total thiol ratio (SH/SH+SS) were calculated [9]. The amounts were expressed as g/dL (albumin), absorbance unit- ABSU (IMA) and umol/L (native and total thiol ratios).

Measurement of the IMA

Albumin Cobalt Binding Test was used to detect the presence of Ischemia Modified Albumin (IMA). This test was performed by adding 50 mL 0.1% cobalt (II) chloride ($CoCl_2,6H_2O$) (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) to the patient serum. After mixing, followed by 10 minutes of incubation, 50 mL 1,5 mg/mL dithiothreitol was added to allow for albumin cobalt binding,. After mixing, followed by 2 minutes of incubation, 1.0 mL of a 0.9% sodium chloride solution was added in order to reduce the binding capacity. The blank was prepared similarly with distilled water instead of dithiothreitol. The absorbance of samples was measured at 470 nm using a spectrophotometer. The results were expressed as absorbance units (ABSU) [10].

Statistical analysis

The Kolmogorov- Smirnov test was used to test the conformity of the measurement data to normal distribution. We applied One way ANOVA test for normally distributed parameters and the Bonferroni test for posthoc values. The results of the tests for normal distribution are given as mean ± standard

derivation (SD). The Kruskal-Wallis test was used to compare the parameters without normal distribution and the Chi- Square test for posthoc values. The results are given as median inter quartile range (IQR). A $p<0.05$ was considered significant for statistical analyses.

Results

A total of 80 children were evaluated in our study. A significant difference was found when the age, height and weight of the patients in the cystitis group (CG) were compared with the control and pyelonephritis groups (PG). When comparing the body temperature of the PG with the control and the CG, significant differences were found. No significant difference was observed between all groups with systolic blood pressure; however, there was a significant difference between control and both PG and CG with diastolic blood pressure. The demographics parameters of the groups are summarized in Table 1.

In our study, significant differences were found between hemoglobin and leukocyte analysis between the control group and pyelonephritis and between the pyelonephritis and cystitis group. There was no significant difference between the groups in terms of platelet values.

Significant differences were obtained in CRP results, between the PG and CG groups and between control and patients groups. In our urine analysis results, there were significant differences in both leukocyte and protein values, when the control group was compared to both groups separately. A significant difference in urine nitrite values when comparing the control group with both groups separately. Table 2 shows the laboratory findings of the groups of our study.

Table 1. Demographics findings of pyelonephritis, cystitis and control groups

Parameteres	Control Group	Pyelonephritis Group	Cystitis Group	p
Age (months)	72 (48-120)	48 (17-84)	105 (87-160)[a,b]	0.0001
Weight (kg)	21 (16-34)	16.5 (13-21)[a]	35 (26-51)[a,b]	0.0001
Height (cm)	119 (102-144)	93 (80-119)[a]	134 (123-153)[a,b]	0.0001
Body temperature (ºC)	36.6 (36.4-36.8)	39 (38.7-39.2)[a]	36.8 (36.5-36.9)[b]	0.0001
Systolic (mmHg)	94 (87.5-100)	100 (92-104)	97.5 (91.3-109)	0.108
Diastolic (mmHg)	58 (49.3-60)	60 (60-69)[a]	65 (60-70)[a]	0.0001

p; Awarded according to the Kruskal-Wallis test. Posthoc evaluations within the groups were done with the Mann-Whitney U test.
a: Significantly different values were given from the control group; b: Significantly different values were given from the Pyelonephritis group.

Table 2. The laboratory findings of pyelonephritis, cystitis and control groups

Parameteres	Control	Pyelonephritis Group	Cystitis Group	p
Hemoglobin (g/dL)	12.5±1.2	11.3±1.3[a]	12.5±1.1[b]	0.001
Leukocyte (µL)	7.4±1.7	16.5±6.3[a]	9.0±2.8[b]	0.0001
Platelets (µL)	308±66.7	336±110	294±48.6	0.207
CRP(mg/L)	0.04 (0.04-0.04)	11.7 (6.3-14.5)[a]	0.04 (0.04-1.2)[a,b]	0.0001*
Urine leukocyte (µL)	0 (0-0)	500 (500-500)[a]	250 (75-500)[a,b]	0.0001*
Urine nitrit (µL)	0 (0-0)	0 (0-1.75)[a]	0.1 (0-2)[a]	0.0001*
Urine protein (mg/dL)	0 (0-0)	40 (0-100)[a]	0 (0-15)[a,b]	0.0001*

p; Awarded according to "OneWay ANOVA test". Posthoc evaluations within the group were done with the Bonferonni test.
*p; Awarded according to the Kruskal-Wallis test. Posthoc evaluations within the group were done with the Mann- Whitney U test.
a: Significantly different values were given from the control group; b: Significantly different values were given from the Pyelonephritis group.

Table 3. The analysis results between thiol / disulphide homeostasis parameters, other characteristic properties, albumin and IMA results of our study population

Parameteres	Control	Pyelonephritis Group	Cystitis Group	p
NativeThiol (µmol/L)	367 (335-403)	362 (285-429)	369 (354-385)	0.659
Total Thiol (µmol/L)	420±69	406±87	427±46	0.615
Disulfid (µmol/L)	24.5±4.7	22.7±6.7	25.4±2.9	0.208
IMA (ABSU)	0.61 (0.57-0.63)	0.61 (0.57-0.68)	0.60 (0.54-0.62)	0.224
Albumin (g/dL)	3.5±0.7	4.1±0.61[a]	3.95±0.56	0.005
Disulfid / NativeThiol (%)	6.6±0.94	6.2±1.12	6.81±0.72	0.183
Disulfid / Total Thiol (%)	5.8±0.73	5.5±0.89	5.98±0.56	0.172
NativeThiol / Total Thiol (%)	88.2±1.47	88.8±1.79	88.0±1.12	0.172

p; Awarded according to "OneWay ANOVA test". Posthoc evaluations within the group were done with the Bonferonni test.
*p; Awarded according to the Kruskal Wallis test. Posthoc evaluations within the group were done with the Mann- Whitney U test.
a: Significantly different values were given from the control group.

Table 3 shows the analysis between thiol/disulfide homeostasis parameters, other characteristic properties, albumin and IMA results of our study population. No statistically significant differences were found in thiol, disulfide and thiol/disulfide ratios and IMA values when comparing all groups. A significant difference was found only with the albumin values when the control group was compared with PG.

We determined a positive correlation of albumin with native thiol, total thiol and disulphide while we determined no correlation of IMA with other parameters in the PG. We determined a positive correlation of both native thiol and total thiol with hemoglobin and albumin, while we determined a negative correlation of disulfide with leukocyte in the CG. We did not determine any correlation of IMA with other parameters in the CG. There was no correlation of CRP with native thiol, total thiol and disulfide ratio in the PG.

Discussion

Here we aimed to determine demographic and laboratory parameters and also the status of dynamic thiol/disulfide homeostasis and correlations using this novel method in pyelonephritis and cystitis in pediatric patients.

In our study, we found significant differences in body temperature, leukocyte, CRP and urine leukocyte values that were significant, especially in pyelonephritis. When we look at the thiol/disulfide homeostasis parameters, we could only obtain positive results in albumin values. When we examine our results in more detail, correlations that were positive in terms of correlation were found in values associated with albumin.

Thiols are mostly found in albumin (the more the albumin, the more the thiol group). We think that we can say that the meaningful results we found in albumin value are indirectly present in thiols. In addition, we were sensitive about the gender, age, height and weight of the patients to be close to each other, so as not to affect the results in our study.

In clinical practice, cystitis and pyelonephritis patients come to the hospital with fever, vomiting, irritability, jaundice and failure to thrive in newborns. Young children (not-toilet-trained) apply with complaints such as irritability, foul-smelling urine, abdominal pain, hematuria and suprapubic tenderness and

older children (toilet-trained) apply with dysuria, foul-smelling or cloudy urine, voiding dysfunction, incontinence, frequency, fever and abdominal or flank pain [2]. DMSA is thought that the most sensitive test for detecting renal scarring and for diagnosis of acute pyelonephritis [2,4]. Previous studies have shown that antioxidants can reduce tissue damage and renal scarring of acute pyelonephritis and dynamic thiol /disulphide balance has an important roles in antioxidant protection [4, 9]. Based on the fact that DMSA is an invasive method, looking at the thiol/disulfide balance, we thought that it might help us in the diagnosis of cystitis or pyelonephritis.

However, we did not detect any changes in this thiol/disulfide hemostasis in our study.

We attributed this to the absence of any evidence of diffuse blood from oxidative stress markers in acute pyelonephritis, or by the fact that these markers were not distinctive parameters in pyelonephritis in the early stages.

In both cystitis and pyelonephritis, the pathology depends on the tissues (bladder/kidney), and the diseases are not systemic. Thiol is an organic compound containing a sulfhydryl (-SH) group, which plays a critical role in preventing the occurrence of any oxidative stress in cells [9, 11, 12]. Dynamic thiol/disulfide balance status plays critical roles in antioxidant defense, detoxification apoptosis, regulation of enzyme activities, transcription and cellular signal transduction mechanisms [9, 11]. In the literature, some groups of diseases in which thiol/disulfide homeostasis is affected have been described. These are diabetes, cardiovascular diseases, cancer, rheumatoid arthritis, chronic kidney disease, acquired immunodeficiency syndrome, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, multiple sclerosis, and amyotrophic lateral sclerosis, liver disorder [9].

Thiol/disulfide balance can be measured by a method developed by Erel et al [9] and after them, Elmas et al showed that this method could be easily performed in a pediatric group of patient studies [13]. They found an association with thiol/disulfide homeostasis and inflammation in obese pediatric patients. Ozyazici et al indicated that dynamic thiol/disulfide balance is shifted towards disulphide formation in patients with acute appendicitis [11]. Kurt et al have found a change in the thiol/disulfide homeostasis with febrile seizure pathogenesis in children [14]. Both acute appendicitis and sepsis are systemic diseases. Ayar et al showed that hemodialysis had a positive effect on thiol/disulfide homeostasis [12]. Again Ayar et al. showed in another study the efficiency of the thiol/disulfide balance with sepsis in children [15].

Both infection and inflammation occur in acute pyelonephritis. Inflammation causes tissue damage. This damage results in released acute phase proteins, cytokines and migration of granulocyte to the affected tissue [16]. Vysakh et al. claimed that the main pathophysiological cause was oxidative stress in acute pyelonephritis [17]. Also, Caliskan et al. found an increase in oxidative stress parameters and antioxidant enzyme activities in a rat model of acute pyelonephritis [18]. Based on the results obtained from these studies, we investigated how the balance of thiol /disulfide homeostasis will be affected in acute pyelonephritis.

This study demonstrated no effective results regarding thiol/disulfide homeostasis in acute pyelonephritis and cystitis diseases. Prospective and randomized controlled trials are needed to confirm the pathological role of thiol/disulfide balance in pyelonephritis and cystitis.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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